PROCESS OPTIMIZATION FOR XYLITOL PURIFICATION USING LIQUID-LIQUID BATCH EXTRACTION: EFFECT OF VOLUME RATIOS AND NUMBER OF STAGES

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ABSTRACT

Xylitol is a five-carbon sugar alcohol that has many significant applications in the food, and pharmaceutical industries owing to its unique properties. Xylitol has proven its core value in reducing tooth decay and as sugar substitute for diabetes patient. In general, purification steps for production of fine product are of great interest in process engineering due to the complexity of the separation processes which leads to the major costing in production. Liquid-liquid extraction is a simple, time and energy saving separation method that employed to separate xylitol and impurities. Objective of this research is to optimize the conditions for purification of xylitol with ethyl acetate as solvent using response surface methodology in batch solvent extraction. Experiment design were done with one factor at time method to screen the significance of various factors, then RSM analysis was performed and experiments was conducted based on the suggested models. All the experiments were done in three replications to minimize systematic and instrument errors. OFAT results had shown that the time of extraction is not significant but the optimum conditions for volume ratio of sample to solvent is 1:5, and the number of multiple staging are 5 stages. Henceforth in RSM analysis, first factor was set to be the volume ratio of sample to solvent ranging from 1:2 to 1:6 and second factor was set to be the number of staging ranging from 2 to 6 stages. RSM analysis results that the overall model is valid, with correlation p-value of 0.002 (<0.05) with both two factors and factors' squared proven to be significant to the response. The lack of fit value of 0.0003 for the model is significant. Feasibility study which is the xylitol extraction optimization using above suggested model by RSM produced from Meranti wood is validated. Results obtained from this research have demonstrated that both volume ratio and number of stages have significant effects in extraction process of xylitol purification and through careful optimization the downstream processing of commercial xylitol could be more effective.

ABSTRAK

Xilitol merupakan sejenis pemanis yang mempunyai 5 karbon dan didapati mempunyai banyak aplikasi dalam industri makanan and farmasi. Xilitol telah dibuktikan bahawa ia mampu mengurangkan kerosakan gigi and digunakan secara am sebagai penganti gula dalam pasaran terutamanya untuk penyakit kencing manis. Secara umum, penghasilan dan proses meningkatkan ketulenan sesuatu bahan domestik merupakan masalah yang utama bagi para jurutera. Kerumitan proses akan membawa kepada kenaikan kos penghasilan. Pengekstrakan cecair merupakan cara yang mudah, jimat masa dan tenaga untuk memisahkan xilitol dan bahan bio-kimia yang tidak dikehendaki. Tujuan kajian ini adalah untuk mengoptimumkan faktor-faktor yang akan mempengaruhi ketulinan xilitol selepas proses pengekstrakan. Xilitol diekstrak dengan menggunakan etil asetat. Semua eksperimen telah dijalankan atas tiga replikasi untuk mengurangkan ralat sistematik dan instrumen. Keputusan OFAT telah menunjukkan bahawa masa pengekstrakan adalah tidak signifikan. Keputusan keadaan optimum untuk nisbah isipadu sampel dan pelarut adalah 1 : 5 , manakala jumlah pelbagai peringkat adalah 5. Julat faktor pertama iaitu nisbah isipadu sampel dan pelarut adalah di antara 1 : 2 hingga 1 : 6 dan julat bagi faktor kedua adalah jumlah peringkat iaitu antara 2 hingga 6 peringkat dimasukkan ke dalam analisis RSM. Daripada keputusan yang terhasil, model keseluruhan adalah signifikan, dengan korelasi nilai-p ialah 0.002 (< 0.05) serta kedua-dua dua faktor dan faktor-faktor ' kuasa dua terbukti signifikan kepada respon . Kajian kebolehlaksanaanpengekstrakan xilitol daripada kayu Meranti menggunakan persamaan daripada keputusan RSM telah dilaksanakan dan ianya adalah sah. Keputusan yang diperolehi daripada kajian ini telah menunjukkan bahawa kedua-dua nisbah jumlah dan bilangan peringkat mempunyai kesan yang penting dalam proses pengekstrakan penulinan xilitol dan pengoptimuman yang teliti perlu diaplikasikan dalam pemprosesan hiliran xilitol secara komersial supaya ketulinan xilitol menjadi lebih berkesan..

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
CCD	Central Composite Design
DF	Degree of freedom
FFD	Full Factorial Design
HPLC	High Performance Liquid Chromatography
LLE	Liquid-liquid extraction
LSD	Least Significant Difference
LSR	Liquid solid ratio
MWCO	Molecular Weight Cutoff
MWS	Meranti Wood sawdust
MWSHH	Meranti Wood sawdust hemicellulosic hydrolysate
OFAT	One-factor-at-time
RI	Refractive Index
RSM	Response Surface Methodology
THF	Tetrahydrofuran
XR	Xylose Reductase

Greek

k	parameter number
Cp	replicate number of central point
α	coded factor
R2	coefficient of determination
ei	residuals
yi	difference between the actual individual values

1 INTRODUCTION

1.1 Background of study

Sweeteners play an important part of the global food and beverage industry and its production require wide range of chemical process technologies owing to the diverse sources and uses. The Polyols sweetener industry is currently in a rapid growth due to the increasing consumer demand for sugar-free and reduced calorie products. The sweeteners that contributes to the surging of this genre of food industry are the sugar alcohols such as xylitol, sorbitol, mannitol ,and maltitol.

Xylitol is a five-carbon polyol with a sweetness similar to sucrose. At 10% solids (w/w) xylitol is isosweet to sucrose. (Munton and Birch 1985). Therefore xylitol is widely manufactured in artificial sugar industry.Xylitol is not only a sugar-free sweetener, but also has unique properties that find applications in pharmaceutical, medical, and in domestic usage. (Gurgel *et al.*, 1995). It is non-cariogenic, shown to be valuable in prevention of dental caries because it is not an effective substrate for plaque bacteria and from various clinical studies ,xylitol caries prevention rates is recorded at least 80 to 85%, compared with the sucrose-using groups. (Makinen et al.,2000)

In addition, xylitol is diabetic tolerance and appears to be a promising agent in prevention of acute otitis media when administered in chewing gum. (Uhari & Tapiainen et al., 2000), Xylitol also prevents osteoporosis and lung infections. (Zabner, 2004) Diabetics and dental caries can be overcome by the substitution of xylitol in sugar contained products. Xylitol can be naturally found in some fruits and vegetables, but extraction of xylitol from these sources is economically not feasible because of the low concentrations present (Saha, 1997).

In industrial scale, xylitol is produced mainly by chemical process that involves the catalytic dehydrogenation of D-xylose that present in lignocellulosic hydrolyzates and the solution produced requires subsequent high-cost purification and separation process to obtain high purity xylitol. (Gurgel et al. 1995) . Nontheless it can be produced via biotechnological method i.e bioconversion of xylose-to-xylitol utilizing microorganisms or enzymes which appears to be economically feasible. In the past decade, yeasts have been studied most widely for the production of xylitol, with extensive emphasis on the genera *Candida* (Barbosa et al., 1988) and *Debaryomyce.* (Converti et al., 2002)

Somehow, the yield and productivity are not promising and breakingthrough purification methods besides chromatographic separation that commonly applied to the separation and recovery of the product. Moreover, the recovery and purification of the product exists as a very complicated step in industrial fermentative processes because of the low product concentration and the complex composition of the fermentation broth as in fermentation processes there are different impurities,such as proteins and carbohydrates, introduced for microbial growth. (Granström & Leisola, 2013)

In general, different separation methods had been utilized and can be used in accordance to the specify needs of the manufacturer, mostly required balancing between processing cost and purity of end product. According to Mussatto et al., ethyl acetate is a promising extractor agent for xylitol purification from the fermented broth, hence is chosen to extract commercial xylitol solution. In present, less focus on LLE compare to membrane separation due to its lower efficiency but LLE is simple, cheap and fast to perform for undergraduate project.

1.2 Motivation

Oral cavity and diabetic is a major health issue globally. Conventional sugars are the main contributor. Xylitol is an artificial sweetener with good taste and approximate 67% reduced calories than conventional sugar that proven to be alternatives for glucose and sucrose. (Heikkila *et al*., 1992).

For daily diets consuming, xylitol does not promote tooth decay and have similar characteristic to both sucrose and dextrose sugars e.g. fructose. (Scheinin et al., 1976) It is not a carbohydrate but a sugar alcohol that can be obtained from the reduction of glucose, changing the aldehyde group to hydroxyl group or by microbial conversion from xylose.

Caries is caused by the dissolution of the teeth by acid produced through metabolism of dietary carbohydrates by oral bacteria. Researchers found out that two main oral bacteria involved in caries formation are of mutans streptococci and lactobacilli. (Aas et al.,2008) These oral bacteria utilized 6-carbon sugar to metabolise, sucrose and dextrose sugars are 6-carbon sugars hence inevitably impact catiogenic effects towards tooth. However, xylitol is a 5-carbon sugar that is non-fermentable by bacteria and therefore cannot act as bacteria energy reservoir, simply thus it has extra anticariogenic effects contributable to against microbial activity, simulation of saliva leading in increased buffer activity and pH increment, and remineralization enchancement. (Trahan,1995) Usage of xylitol for prolonged period is strongly believed that can reduce the caries and ever since Turku Sugar Studies back in year 1975, several laboratory studies had been carried out consistently (Makinen K.K.,2000)

Another dominant issue caused by high usage of conventional sugars in diet is diabetes.Diabetes is a group of metabolic diseases caused by the insulin inefficiency which results in high blood glucose concentration. Observations concluded that following the ingestion of xylitol, the blood glucose and insulin response are remarkedly lower than glucose or sucrose ingestion hence is a suitable sweetener for use in diabetics controlled diets. (Lyengar et al.,1985)

These are due to xylitol poor metabolism rate relative to glucose and sucrose ingestion, slow xylitol conversion rate to glucose that take place in the hepatocytes and slow absorption rate into bloodstrea, and the lack of a specific transport system across the intestinal mucosa with only 25% to 50% xylitol is absorbed via passive diffusion varying on several factors. (Demetrakopoulos and Amos,1978). Therefore xylitol usage as sugar substitute does not significantly affects blood glucose concentration and serum insulin responses in our body.

In view of the above benefits from xylitol, the manufacture of xylitol had grown substantially. Many purification technique has been employed ever since, till date the main challenge was the technique used, i.e. anion and cation exchange resins to purify xylitol from sugarcane bagasse hydrolysate fermentation broth (Gurgel et.al.,1995) that yield high xylitol loss and tend to be high cost for industrial scale processes. In order to overcome this impediment, an efficient and economically competitive strategy for xylitol purification and recovery was established. The purification of solutions by liquid–liquid extraction is used in various industrial processes in order to recover dissolved substances or to remove undesirable impurities.

The motivation of conducting this study is hence to remove undesirable impurities and recover dissolved xylitol using liquid-liquid extraction that is widely used in many industrial processes and suggested by previous work of Mussato and coworkers that optimizing the extraction parameters will enhance the extraction of impurities.To date the literature of phase diagram and kinetic study for each specific system are usually not available for bio-based compound e.g. sugars (A. Acrivos et al. ,2002) hence the purification procedure has to be experimentally optimized which formed the problem statement of this research.

Henceforth, in this research liquid-liquid extraction was employed to purify the commercial xylitol and later microbial enzyme-reduced xylitol by batch. In this study, Ethyl acetate was chosen as solvent based on previous work results. (Mussato et.al., 2005)

1.3 Problem statement

Xylitol recovery methodology has become the main research focus as compared to its production in recent year owing to its various commercial application. There is a need of large scale production that essentially cost-effective and environmental friendly. For commercial product often high purity is required, in order to recover a product with complex chemical structure many steps come after. (A. Acrivos et al. ,2002). Therefore studies are needed in order to determine the most optimum conditions that can enhance the separation efficiency on purification of xylitol using the most common laboratory scale method, liquid extraction.

1.3.1 Significance of Study

Purification of solutions by liquid-liquid extraction is widely used in many industrial processes to recover dissolved substances or to remove undesirable impurities. Its advantages are simple, clean and fast. Solvent extraction also is widely used due to their low boiling point that easy to control and cost-efficient.. The greater the removing of impurities give rise to the higher quality of fine xylitol hence increases its economic value. To date the literature of phase diagram and kinetic study for each specific system are usually not available to provide insight of the purification process thus the purification procedure has to be experimentally optimized which leads to the need of this research. The purification strategy selected was then optimized for maximum xylitol purified.

1.4 Objective

Liquid-liquid extraction was chosen as a methodology to purify xylitol from the commercial xylitol solution. This research aims to evaluate the following parameters that affects the purification process., ratio of xylitol solution to ethyl acetate , initial concentration of xylitol, and the time of extraction process.

The specific objectives of this research are:

1. To study the effect of optimum conditions to the purity of xylitol after liquid-liquid extraction.

2. To optimize the controlled parameters to the purity of xylitol after extraction using response surface methodology.

3. To validate the results for the commercial xylitol purification process by conducting feasibility study on purification of xylitol from biomass.

1.5 Research questions

1. What are the optimum process conditions for the single stage of liquid-liquid extraction to the effect of the purity of the xylitol after extraction?

2. How many stages are required to extract the xylitol in a significant amount?

3. How much resemblance of feasibility study (enzymatic production) to commercial study?

1.6 Scopes of Study

This research study will be done using liquid extraction with separation funnel and ethyl acetate as solvent.

i. Three main parameters will be monitored in the separation process experiment to identify the most influential factors that affects the composition of xylitol at the extracted layers using Central Composite Design in Design Expert 7.0 software, which are : volume ratio of xylitol solution to ethyl acetate, extraction stages, and the time of extraction process.

ii. The range of significance of all the parameters will be determined by one variable at time (OFAT).

iii. The optimization of the correlation of all parameters will be done using Response Surface Methodology (RSM) in Design Expert 7.0.

iv. The xylitol purities before extraction and after extraction will be analyze using High Performance Liquid Chromatography (HPLC) by comparing the RI values with the standard.

1.7 Organisation of this thesis

Chapter 1 comprises of the introduction part of this thesis which discussed on the background of the xylitol and of this study. The current issues encounter by industrial on xylitol downstream processing and its potential solutions were discuss briefly and lead to the motivation of this research study. Next, problem statement, research questions and scopes of study were clearly listed lastly the specific objectives to achieve for this research were also included.

Chapter 2 was the review part of this thesis supported by citated literature. Firstly, the history and the properties of the xylitol that needed to be mastered were presented. Then the importance of xylitol i.e. its applications were briefly discussed. Review on upstream production of xylitol were disserted. Downstream process for xylitol purification were reviewed from different previous studies and summarization of upstream and downstream were discussed in table form. Then the literature review for the process i.e. liquid-liquid extraction, analysis i.e. HPLC and statistic and optimization i.e. RSM were included in this chapter.

Chapter 3 give insight for the methodology part in this thesis comprises of chemical used in this research follow by the experimental flowchart and the study operational framework diagram. Next, the liquid-liquid extraction set up were explained in detailed. Standard and sample preparation were explained associated with standard curve generated. The experimental design of utilizing OFAT method were detailed out in sequence and the procedure to perform statistical analysis and optimization of factors affecting the response were explained. Lastly the procedure on analysis using HPLC and the XR production methodology were written correspondingly.

In Chapter 4, results from the commercial xylitol extraction were tabulated. Results from OFAT were presented in graphical manners and discussion on the trend and justifications were made appropriately. Next, result from RSM analysis divided into two part: statistical analysis part to check on experimental validity and optimization part, to check on the interaction and degree of affects to the response were tabulated with diagrams and result generated from Design Expert software. Lastly, the result for feasibility study and fermentation of xylose using XR enzyme were presented. Discussions were made on every result collected from this experimental study.

Chapter 5 were the conclusion of this research to ensure that the research objectives were met and several recommendations were given for future use.

2 LITERATURE REVIEW

2.1 Overview of the project

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2.2 History of xylitol

In September, 1890, the German chemist expert Emil Herman Fischer and his assistant, Rudolf Stahel, separated a new compound from beech chips which was named Xylit, the German word for xylitol (Fischer and Stahel, 1891). Later, in 1902, owing to his outstanding chemical accomplishments, Dr. Fischer was awarded the Nobel Prize in chemistry. Almost simultaneously with Fischer, the French chemist M.G. Bertrand had managed to isolate xylitol syrup in processing wheat and oat straw (Bertrand, 1891).

After five decades, xylitol was first found in low concentration in some plants and was widely used during World War II as sugar substitute due to global food shortage. During the 1950s, Dr. Oscar Touster's work has changed the xylitol value remarkably. He and his co-workers had concluded that xylitol is formed in the human body and its metabolism is associated with pentosuria from investigations on L-xylulose. Dr. Touster reasoned that essential pentosuria involved the accumulation and excretion of a metabolite which is readily disposed of in normal, but not in pentosuric individuals. Eventually, the product was isolated and characterized as xylitol (Touster and Shaw, 1962).

Later in year 1962 a biochemical pathway involving xylitol was discovered in mammalian tissue gave rise to the intensive studies on xylitol as a natural sweetener. In 1970 the first study on the effects of xylitol on dental plaque was started in Turku, Finland. Its clinical applications were then confirmed after publication of Turku studies in 1975 (Scheinin and Makinen, 1975).

The first commercial xylitol chewing gums (XyliFresh) were launched in Finland and in the United States on the same year. Nowadays, variety of products manufactured from xylitol, scientists investigate more potential health benefits, while engineers develop more efficient and cheaper production methods.

2.3 Properties of xylitol

Xylitol (Figure 2.1.) is a five-carbon polyol with sweetness similar to sucrose and unique chemical and physical properties contribute to its increasing growth demand.

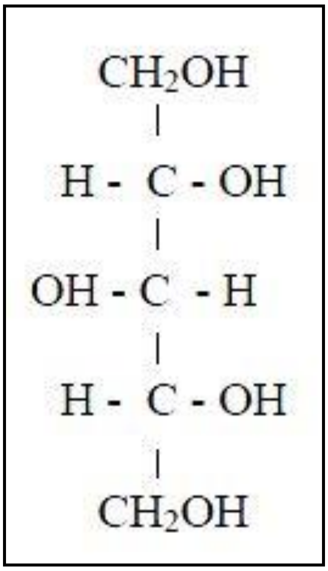

Figure 2.1 : Chemical structure of xylitol

There are many sugar polyol in the artificial sweetener market e.g. sorbitol, mannitol, erythritol and xylitol etc. Sweetness remains as the major factor for the appealing of artificial sugar production to ensure that end consumer acceptance of product taste and quality is secured. Among all, xylitol has found out to be the sweetest alternative sugar-free product with sweetness similar to sucrose (Figure 2.2). . At 10% solids (w/w) xylitol is isosweet to sucrose (Munton and Birch 1985).

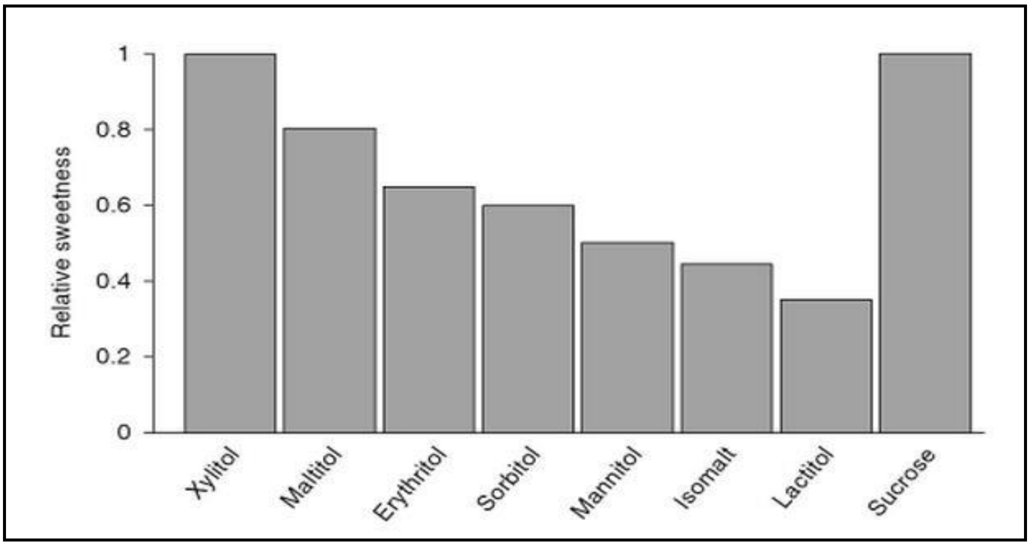

Figure 2.2 : Relative sweetness of Polyols (Moskowitz, 1971)

Among polyol, xylitol is an imperative sugar substitute with interesting physical and chemical properties which make it a high value compound for pharmaceutical, odonatological and food industries. (Table 2.1)

Table 2.1 : Physical properties of xylitol (Nabors, 2012)

Common Name	**Xylitol**
Formula	$C_5H_{12}O_5$(Molecular Weight 152.15)
Appearance	White, crystalline powder
Odor	None
Specific rotation	Optically inactive
Solubility at 20 °C	169 g/100 g H_2O
pH in water (100 g/liter)	5 – 7
Density (bulk density) (15 °C)	1.50 g/L
Density (specific gravity) of aqueous solution (20 °C)	10% 1.03g/ml; 60% 1.23g/ml
Viscosity (cP) (20 °C)	10% 1.23; 40% 4.18; 50% 8.04; 60% 20.63
Melting Point (°C)	92 – 96
Boiling Point (at 760 mmHg)	216 °C
Caloric value	4.06 cal/g (16.88 J/g)
Heat of solution	endothermic +34.8 cal/g (145.65J/g)
Heat of combustion	16.96kJ/g
Refractive index (25 °C)	10% 1.3471; 50% 1.4132
Moisture absorption (4days, RT)	60% RH, 0.051% H_2O; 92% RH, 90% H_2O
Relative sweetness	Equal to sucrose; greater than sorbitol and mannitol

Xylitol is found naturally in fruits, berries and vegetables (Table 2.2) and is produced 5-15g/day in the human body during normal metabolism in the liver (Touster, 1974). Xylitol can be extracted from fruits and vegetables, however, due to the low concentrations; the extraction is not economical (Saha, 1997). Xylitol can be produced by microbial reduction of D-xylose or catalytic chemical reduction of xylan-rich hemicellulose hydrolysates. Xylan-rich substrates are birch wood, oats, corn fibre, cotton-seed hulls, sugar cane bagasse, rice straw and nut shells, which could be used for xylitol production.(Counsell, 1978)

Table 2.2 : Natural occurrence of xylitol in fruits and vegetables (Washuett J *et al.*, 1973)

Product	**Xylitol content (mg/100g dry solids)**
Yellow plums (*Prunus domestica ssp.italia*)	935
Strawberry (*Fragaria var.)*	362
Cauliflower *(Brassica oleracea var.botrytis)*	300
Raspberries *(Rubus idaeus)*	268
Endives *(Cichorium endivia)*	258
Bilberry *(Hippophae rhamnoides)*	213
Aubergine *(Solanum melongena)*	180
Lettuce *(Lactuca sativa)*	131
Spinach *(Spinacia oleeracea)*	107
Onions *(Allium cepa)*	89
Carrot *(Daucus carota)*	86

Example of chemical composition analysis by several researchers in corn fibres hydrolysate (Table 2.3)

Table 2.3: Chemical composition of corn fibre hydrolysates

Hydrolysate	Xylose (%)	Glucose (%)	Arabinose (%)	Galactose (%)	References
Corn Fibber	17	37	11	4	Saha and Bothast (1999)
	30-41	25-38	21-28	4-6	Hespell (1998)
	20	20	10	-	Leathers (1996)
	20	16	11	2	Dien (1999)